



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 9/96, 11/08, C07K 17/08, C11D 3/386, A61K 7/48, 47/48</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/30148</b> <b>(43) International Publication Date:</b> 21 August 1997 (21.08.97)
<b>(21) International Application Number:</b> PCT/DK97/00051 <b>(22) International Filing Date:</b> 7 February 1997 (07.02.97) <b>(30) Priority Data:</b> 0154/96 15 February 1996 (15.02.96) DK <b>(71) Applicant (for all designated States except US):</b> NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BISGÅRD-FRANTZEN, Henrik [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). OLSEN, Arne, Agerlin [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). PRENTØ, Annette [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). <b>(74) Common Representative:</b> NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> CONJUGATION OF POLYPEPTIDES		
<b>(57) Abstract</b> <p>The present invention relates to polypeptide conjugates with reduced allergenicity comprising a polymeric carrier molecule having two or more polypeptide molecules coupled thereto. It is also the object of the invention to provide a process for producing said polypeptide conjugates with reduced allergenicity, compositions comprising a such polypeptide conjugate with reduced allergenicity, the use of said polypeptide conjugate for a number of industrial applications, such as the use in personal care products and in detergent compositions.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

Title: Conjugation of polypeptides

#### FIELD OF THE INVENTION

The present invention relates to a polypeptide conjugate with  
5 reduced allergenicity, a process for producing said polypeptide  
conjugate with reduced allergenicity, compositions comprising  
said polypeptide conjugate with reduced allergenicity, the use  
of said polypeptide conjugate for reducing the allergenicity of  
a vast number of industrial products and the use in a number of  
10 applications, such as the use in personal care products and in  
detergent compositions.

#### BACKGROUND OF THE INVENTION

An increasing number of polypeptides, including proteins and  
15 enzymes, such as proteases, are being produced industrially by  
microorganisms for use in industry, household, food/feed, cos-  
metics or medicine etc. Said polypeptides may under certain  
circumstances inflict a potential risk to especially employees  
handling the manufacturing of products containing polypeptides,  
20 and also to some extent to users of these products, such as  
hairdressers, and end-users of cosmetic and toiletry products  
etc.

This potential risk need to be controlled and/or limited.

25

#### Allergenicity of polypeptides

In general polypeptides are potential antigens toward which the  
human immune system can produce specific antibodies upon expo-  
sure. This process is known as "immunization" when a clinical  
30 beneficial response is obtained whereas the term "sensitiza-  
tion" is applied when the response leads to hypersensitivity.  
During the primary exposure clonal selection and expansion of  
the specific B-cell clones are initiated, meaning that a pro-  
tective or allergic response will only be a clinically manifest  
35 upon following exposures. The allergic reaction can be defined

as an pathological immune response elicited by otherwise un-harmful agents in low concentrations. The process of sensitisation leading to type I hypersensitivity are characterized by the formation of specific IgE antibodies. At present, the mechanism controlling the subclass shifting are not fully understood.

IgE secreted from activated B-cells can attach to Fcε receptors located on the surface of mast cells and basophil granulocytes, which contain numerous cytoplasmic granules packed with chemical mediators e.g. histamine (J. Klein, "Immunology", Blackwell Sci. Pub., London, 1990; E. Benjamini & S. Leskowitz, "Immunology", Wiley-Liss, N.Y. 1991).

In atopic individuals each of these cells can have a high number of IgE molecules bound to its surface, where they can remain available to interact with allergens for weeks. Upon contact with an allergen the surface bound IgE crossbinds the allergen, leading to the release of cytoplasmic granules into the proximity of the cell, thereby causing the inflammatory allergic reaction.

The role of IgE has been shown to relate to natural immunologic defence systems towards parasitic worms infections and the development of allergies has been suggested to be an unfortunate by-product of this defence system.

The natural allergens causing IgE mediated hypersensitivity can be classified according to their way of exposure: Inhalant allergens (pollens, dust mites etc.), Ingested allergens (milk, eggs etc.); Contact allergens (e.g. from latex) and allergens from stinging insects (e.g. bees, fire ants etc.). The aero-allergens represents clinically by far the largest group, stressing an area of high potential risk for the industrial polypeptides.

Testing for allergy can either be performed as *in vivo* provocation, most commonly skin prick testing of by a number of *in vi-*

tro assays, primarily based on IgE levels in peripheral blood. In spite of the great efforts in the latter area the most reliable way to diagnose allergy is still the *in vivo* challenging, which again has different levels of sensitivity depending  
5 on the selected target organ.

For instance, intranasal challenge with allergenic proteins can provoke an allergic response even though skin tests and radio-allergosorbent test (RAST) for specific serum IgE are negative  
10 (Ivan Roitt, "Essential Immunology", fifth edition, p. 152 and p. 240, 1984).

#### Reduction of allergenicity of polypeptides

Presently, the generation of allergic responses to industrial  
15 polypeptides is avoided by immobilizing, granulating, coating or dissolving the products, especially to avoid the formation of airborne material. Anyhow, these methods still represent a risk of dust or aerosol formation during handling and processing, with the subsequent risk of allergic sensitisation.

20 There will, anyhow, still be a risk of having polypeptide dust or dissolved polypeptide in aerosol form. Therefore some release of enzymes can occur leading to a possible sensitisation and subsequent allergic response.

25 Another way of diminishing the problem has been to select polypeptides of human origin for production, e.g. in bacteria, fungi, yeast, or mammalian cell cultures. Furthermore, it will in many cases not be possible to find polypeptides of human  
30 origin with the desired properties, wherefore other origin has to be considered. This can be either human polypeptides that are altered in one or more positions in the molecule, giving the performance that is desired. It might also be molecules from other species, including bacteria, mold etc. All the  
35 latter groups of products will have potency for immune stimulation in mammals.

A further proposition for decreasing allergenicity has been to reduce the size of the protein molecules (see e.g. JP Patent Publication No. 4,112,753, or Research Disclosure No. 335,102). This is, however, a solution that is only available when the activity of the protein is without importance, or in such rare cases, where the activity of the protein is retained in spite of a breakdown of the protein.

The application of protein engineering has been suggested to reduce the allergenicity of proteins through epitope mapping and subsequent change of the allergenic epitopes (see WO 92/10755 (Novo Nordisk A/S). This procedure usually requires a large investment in work and development.

Another technology which can be used for reducing the immune system's response towards polypeptides is the "PEGylation"-technology, which involves modification of polypeptides by means of covalent attachment of strands of polyethylene glycol (PEG), to polypeptide molecules. This technique have been known for more than 20 years (see e.g. US patent no. 4,179,337), but is today only used in connection with polypeptides for pharmaceutical use. Consequently the main purpose is to reduce the immune system's production of IgM and/or IgG.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the number of Dunkin Hartley guinea pig having been exposed to 1.0 µg monomer Peroxidase and 1.0 µg Dextran-Peroxidase A and B intratracheally found to be enzyme specific positive vs. weeks starting from the day of exposure.

Figure 2 shows the number of Dunkin Hartley guinea pig having been exposed to 1.0 µg lipase and 1.0 µg Dextran-lipase intratracheally found to be enzyme specific positive vs. weeks starting from the day of exposure.

Figure 3 shows the number of Dunkin Hartley guinea pig having been exposed to 1.0 µg cellulase and 1.0 µg Dextran-cellulase

intratracheally found to be enzyme specific positive vs. weeks starting from the day of exposure.

#### SUMMARY OF THE INVENTION

5

It is the object of the present invention to provide polypeptide conjugates with reduced allergenicity.

10 It is to be understood that, in connection with industrial applications of polypeptides, it is mainly the inhalation of the allergens that may inflict a risk of an allergic response. Therefore, one of the crucial advantages of the present invention is that the inventors have solved the problem of the respiratory challenge with allergens, whereas prior art solutions  
15 mainly concern dermal challenge with alleged immunogens. The respiratory challenge is a much more sensitive question.

A few important terms essential for the understanding of the present invention will be defined in the following, as these  
20 terms are often used in connection with the description the immune systems response towards polypeptides, in a unclear manner, even by many scientists.

#### Immunogenicity, antigenicity and allergenicity

25 "Immunogenicity" is a wider term than "antigenicity" and "allergenicity" and expresses the immune systems response to the presence of foreign substances. Said foreign substances are called "immunogens", "antigens" and "allergens", respectively, depending on the type of immune response they elicit.

30

An "immunogen" is a substance which, when introduced into circulatory system of animals and humans, is capable of stimulating an immunologic response.

35 The term "antigen" refers to substances which by themselves are capable of generating antibodies when recognized as a non-self molecule.

An "allergen" is an antigen which gives rise to allergic sensitization or an allergic response due to the formation of IgE antibodies (in humans, and molecules with comparable effects in animals).

The above used term "circulatory system" of the body of humans and animals means in the context of the present invention the system which mainly consist of the heart and blood vessels. The heart delivers the necessary energy for maintaining blood circulation in the vascular system. The circulation system functions as the organisms transportation system, transporting (in the blood) O<sub>2</sub>, nutritious matter, hormones, and other substances of importance for the cell regulation into the tissue. Further, the blood removes CO<sub>2</sub> from the tissue to the lungs and residual substances to e.g. the kidneys. Furthermore, the blood is of importance for the temperature regulation and the defence mechanisms of the body, including the immune system.

In the context of the present invention a polypeptide conjugate having "reduced allergenicity" indicates that the amount of produced IgE (in humans, and molecules with comparable effects in specific animals), which might lead to an allergic state, is significantly decreased when inhaling a polypeptide conjugate of the invention in comparison to the corresponding parent polypeptide molecule.

As mentioned above it is, at least in the context of polypeptides of the present invention, important to distinguish between dermal allergens mediating allergic responses caused by skin contact, and respiratory allergens causing allergic responses by contact with cell-bound IgE in the bronchial tree, due to the well-known fact that skin tests may be negative even though inhalation tests provoke an allergic response.

Therefore, assessment of allergenicity may be made by inhala-



tion tests, comparing the effect of intratracheal administrated parent polypeptides with the corresponding polypeptides of the invention with reduced allergenicity.

- 5 A number of *in vitro* animal models exist for assessment of the allergenicity of polypeptides. Some of these models give a suitable basis for hazard assessment in man. Suitable models include a guinea pig model and a rat model. These models seek to identify respiratory allergens as a function of elicitation  
10 reactions induced in previously sensitised animals. According to these models the alleged allergens are introduced intratracheally into the animals.

- A suitable strain of guinea pigs, the Dunkin Hartley strain,  
15 does not as humans, produce IgE antibodies as the allergic response. However, they produce another type of antibody the IgG1A and IgG1B (see e.g. Prentø, ATLA, 19, p. 8-14, 1991), which are responsible for their allergenic response to inhaled polypeptides including enzymes. Therefore, when using the Dun-  
20 kin Hartley animal model, the relative amount of IgG1A and IgG1B is a measure of the allergenicity level.

- A rat strain suitable for intratracheal exposure to polypeptides and enzymes is the Brown Norway strain. The Brown Norway  
25 strain produces IgE as the allergic response.

Other animals such as rabbits etc. may also be used for comparable studies.

- 30 In Example 1 the surprising discovery of the present invention is disclosed showing that the allergenicity of purified wild-type *Coprinus cinerea* peroxidase coupled to dextran ( $M_r$  about 1,000 kDa) intratracheally introduced into Dunkin Hartley guinea pigs is reduced in comparison to the corresponding  
35 monomer peroxidase ( $M_r$  about 39 kDa).

Example 2 and 3 show that also the allergenicity of lipase and

cellulase, respectively is reduced.

In the first aspect the invention relates to a polypeptide conjugate with reduced allergenicity which comprises one  
5 polymeric carrier molecule having two or more polypeptide molecules coupled covalently thereto.

The polypeptide molecules may be coupled to the polymeric carrier molecule using any methods. It is well-known by a  
10 person skilled in the art to couple chemical groups on the polypeptides, such as free hydroxyl groups, free sulfhydryl groups, free acid groups, free amino groups, to hydroxy groups of a vast number of polymeric (carrier) molecule.

15 In an embodiment of the invention the polymeric carrier molecule is coupled with two or more polypeptide molecules via a covalent linkage formed between one of the two vinyl groups of a divinyl sulfone.

20 The term "polypeptides" means in the context of the present invention all types of polypeptides such as proteins, enzymes, ligands, antibodies, inhibitors, receptors which may constitute a greater or less part of a commercial product. Especially contemplated are polypeptides which, as ingredients in a commercial  
25 product, are not to be introduced into the circulatory system of the body of humans or animals.

In the second aspect the invention relates to a process for producing polypeptides with reduced allergenicity comprising  
30 the steps of

- i) activating a polymeric carrier molecule, and
- ii) reacting two or more polypeptide molecules with said activated polymeric carrier molecule under conditions suitable for conjugation, and
- 35 iii) blocking of residual active groups on the conjugate.

In a preferred embodiment of the process of the invention the

polymeric carrier molecule is coupled directly with two or more polypeptide molecules or via a reactive linker molecule.

5 The activation of the polymeric carrier molecule in step i) may be performed by any method known in the art. Examples of such suitable coupling methods will be described below.

10 In an embodiment of the invention two or more polypeptide molecules are coupled to the polymeric carrier molecule via a divinyl sulfone.

The third object of the invention is to provide compositions comprising a polypeptide conjugate of the invention.

15 Such compositions may further comprise polypeptides, such as proteins and/or enzymes and/or ingredients normally used in e.g. products such as detergents, household article products, agrochemicals, personal care products, cosmetics, toiletries, oral-, skin and hair care products, composition use for pro-  
20 cessing textiles, compositions for cleaning hard surfaces, compositions used for manufacturing food, feed, juice, wine and beverages, and also oral and dermal pharmaceuticals.

25 The above group of products is sometimes referred to as "industrial products". This term will be used below to describe the group of products which are especially contemplated according to the present invention.

30 In the final aspect the invention relates to the use of polypeptide conjugates of the invention for a number of applications for industrial products, such as personal care applications and the use in detergent compositions.

#### DETAILED DESCRIPTION OF THE INVENTION

35

It is the object of the invention to provide polypeptide conjugates with reduced allergenicity.

As described above it is well-known according to prior art to conjugate one or more polymeric molecules to one polypeptide carrier molecule by covalently attaching one or more of poly-  
5 meric molecules to e.g. the amino-groups of a polypeptide carrier molecule. Even though techniques for doing so have been shown to reduce the immune system's response towards polypeptides said techniques have some deficiencies:

10 - As the number of accessible attachment-groups (e.g. amino groups) on many polypeptide molecules, used as ingredients in industrial products (i.e. normally polypeptide molecules with a molecular weight in the range from about 10 to 200 kDa), are limited, the number of polymeric molecules which can be conju-  
15 gated to each polypeptide carrier molecule is limited.

- the activity (e.g. catalytic activity) of polypeptide conjugates (e.g. enzyme conjugates) having a number of polymeric molecules attached to each polypeptide carrier molecule is  
20 usually significantly decreased in comparison to the corresponding parent polypeptide molecule, due to steric hindrance (i.e. low accessibility of the substrate to the active site of the polypeptide molecule, e.g. enzyme).

25 The inventors have found that conjugation techniques resulting in the coupling of two or more polypeptide molecule to each polymeric carrier molecule reduces the allergenicity of polypeptide molecules suitable for industrial products.

30 Such polypeptide conjugates are also more stable than corresponding parent polypeptide molecules.

Polypeptide conjugates according to the present invention may also have advantages in comparison to polypeptide conjugates  
35 having several polymeric molecules attached to each polypeptide carrier molecules, as

- the amount of excess polypeptide molecules needed for the conjugation process is less than the amount needed for corresponding prior art processes conjugating several polymeric molecules to each polypeptide carrier molecule, as only one attachment-group on the polypeptide molecule is needed.

- the activity (e.g. catalytic activity) of polypeptide conjugates of the invention is maintained to a greater extent than corresponding prior art polypeptide conjugates having several polymeric molecules attached to each polypeptide carrier molecules, as the steric hindrance is less.

#### Conjugates of the invention

Accordingly, in the first aspect the invention relates to polypeptide conjugates with reduced allergenicity which comprise one polymeric carrier molecule having two or more polypeptide molecules coupled covalently thereto.

The two or more polypeptide molecules may be coupled to the polymeric carrier molecule by any method known in the art.

In an embodiment of the invention said conjugate comprises a polymeric carrier molecule having coupled thereto, via a covalent linkage, formed between one of the two vinyl groups of a divinyl sulfone, one or more polypeptide molecules.

The total molecular weight ( $M_r$ ) of said polypeptide conjugate lies in the range between 50 kDa and 40,000 kDa, preferably between 100 kDa and 1000, especially 200 kDa and 500 kDa.

Further, conjugates according to the invention have between 1 and 60, preferably 2 and 40, especially 3 and 20 polypeptide molecules coupled to each polymeric carrier molecule.

It is within the scope of the invention to couple two or more different polypeptide molecules to each polymeric carrier molecule. The different polypeptides may exhibit different activi-

ties, such as two different enzyme activities. Further, said polypeptide molecules may also be functionally different.

5 Examples of functionally different polypeptide molecules include enzyme molecules, ligand molecules, inhibitor molecules, receptor molecules and antibody molecules.

10 Further, it is also possible to provide conjugates having coupled thereto e.g. an enzyme, such as an oxidase and an effector molecule (i.e. an enhancer). Due to the proximity of said two molecules a very effective conjugate can be obtained.

#### The polymeric carrier molecule

15 The polymeric carrier molecule to which the polypeptide molecules are to be coupled may be any polymer with more than two attachment groups. This includes natural or synthetic homopolymers, such as polyols (i.e. poly-OH), polyamines (i.e. poly-NH<sub>2</sub>) and polycarboxyl acids (i.e. poly-COOH), and further heteropolymers i.e. polymers comprising at least two different  
20 attachment groups e.g. a hydroxyl group and amine groups.

The synthetic homo- and heteropolymeric carrier molecules include Star-PEGs, Branched PEGs, poly-vinyl alcohol (PVA), polycarboxylates, poly-(vinylpyrrolidone) and poly-D,L-amino acids.

25 Star-PEGs are multi-armed polyethylene glycol molecules made by polymerization of ethylene oxide molecules from a crosslinked divinyl benzene core (Gnanou et al. (1988), Makromol. Chem. 198, 2885; Rein et al (1993), Acta Polymer, 44, 225). Star-PEGs  
30 and also Branched PEGs are available from Shearwater Inc., USA).

35 Examples of suitable naturally occurring homo- and heteropolymers comprise dextrans including carboxymethyl-dextrans, celluloses such as methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose and hydroxypropylcellulose, hydrolysates of chitosan, starches, such as hydroxyethyl-

starches and hydroxypropyl-starches, glycogen, agarose, guar gum, inulin, pullulan, xanthan gum, carrageenin, pectin, alginic acid etc.

- 5 In an embodiment of the invention the molecular weight of the polymeric carrier molecule lies between 1 kDa and 10,000 kDa, preferably between 2 kDa and 5,000 kDa, especially between 5 kDa and 500 kDa.
- 10 Note that all polymer molecular weights mentioned in connection with the present invention are average molecular weights.

The polypeptide molecule

- 15 The polypeptide molecules to be coupled to the polymeric carrier molecule may be any type of polypeptide molecules. However, it is preferred that said polypeptide molecule have some sort of functionality.

- 20 The polypeptide to be modified according to the invention may be of plant, animal or microbial origin, although the polypeptide molecules of microbial origin, such as of bacterial or fungal origin (i.e. originated from filamentous fungi or yeasts) are preferred.

- 25 Especially contemplated polypeptide molecules are proteins having either an anti-microbial, biological or enzymatic activity.

- 30 In the case of the protein being an enzyme it may be an enzyme from the group including hydrolases, such as proteases, lipases and cellulase, transferases, carbohydrases, oxidoreductases, such as laccase and peroxidase, or phytases.

- 35 Most enzymes used in industrial products have a molecular weight in the range from about 4 kDa to 200 kDa, preferably 15 kDa to 150 kDa, especially 20 to 100 kDa.

Ligands contemplated according to the invention will in most case have a molecular weight in the range from 100 dalton to 2,000 dalton.

- 5 The inventors have found that the enzymatic activity of enzyme conjugates of the invention are substantially maintained.

10 A "substantially" maintained activity is in the context of the present invention defined as an activity which is at least between 20% and 30%, preferably between 30% and 40%, more preferably between 40% and 60%, better from 60% up to 80%, even better from 80% up to about 100%, in comparison to the activity of the parent unmodified polypeptide molecule.

15 The process of the invention

In the second aspect the invention relates to a process which is suitable for large scale processing of polypeptide molecules to obtain polypeptide conjugates with reduced allergenicity.

- 20 According to the invention one or more polypeptide molecules are coupled to each polymeric carrier molecule by
- i) activating said polymeric carrier molecule, and
  - ii) reacting one or more polypeptide molecules with said activated polymeric carrier molecule under conditions suitable
  - 25 for obtaining conjugation, and
  - iii) blocking of residual active groups on the conjugate.

The activation of the polymeric carrier molecule in step i) may be performed by any method known in the art. Examples of such

30 methods are described in the following.

Activation of the polymeric carrier molecule

Methods and chemistry for activation of polymeric molecules as well as for conjugation of polypeptides are intensively described in the literature. Commonly used methods for activation

35 of insoluble polymeric molecules include activation of functional groups with cyanogen bromide, periodate, glutaraldehyde,



biepoxides, epichlorohydrin, divinylsulfone, carbodiimide, sulfonyl halides, trichlorotriazine etc. (see R.F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S.S. Wong, (1992), "Chemistry of Protein  
5 Conjugation and Crosslinking", CRC Press, Boca Raton; G.T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.). Some of the methods concern activation of insoluble polymeric molecules but are also applicable to activation of soluble polymeric molecules e.g. periodate,  
10 date, trichlorotriazine, sulfonylhalides, divinylsulfone, carbodiimide etc. The functional groups being amino, hydroxyl, thiol, carboxyl, aldehyde or sulfydryl on the polymeric molecule and the chosen attachment group(s) on the polypeptide molecule(s) must be considered when choosing the activation and  
15 conjugation chemistry.

Techniques involving coupling electrophilically activated polyols to the amino groups of lysins can also be useful. Many of the usual leaving groups for alcohols give rise to an amine  
20 linkage. For instance, alkyl sulfonates, such as tresylates (Nilsson et al., (1984), Methods in Enzymology vol. 104, Jacoby, W. B., Ed., Academic Press: Orlando, p. 56-66; Nilsson et al., (1987), Methods in Enzymology vol. 135; Mosbach, K., Ed.; Academic Press: Orlando, p. 65-79; Scouten et al., (1987),  
25 Methods in Enzymology vol. 135, Mosbach, K., Ed., Academic Press: Orlando, 1987; p. 79-84; Crossland et al., (1971), J. Amr. Chem. Soc. 1971, 93, p. 4217-4219), mesylates (Harris, (1985), JMS-REV. Macromol. Chem. Phys. C25, 325-373; Harris et al., (1984), J. Polym. Sci. Polym. Chem. Ed. 22, p. 341-352),  
30 aryl sulfonates like tosylates, and para-nitrobenzene sulfonates can be used.

Organic sulfonyl chlorides, e.g. Tresyl chloride, effectively converts hydroxy groups in a number of polymeric molecules into  
35 good leaving groups (sulfonates) that, when reacted with nucleophiles, like amino groups in the polypeptide chain, allow stable linkages to be formed between the polymeric molecule and

the polypeptide molecules. In addition to high conjugation yields, the reaction conditions are in general mild (neutral or slightly alkaline pH, to avoid denaturation and little or no disruption of activity), and satisfy the non-destructive requirements of the polypeptide molecules.

Oxirane groups and other epoxide groups may also been used for creating amine bonds.

10 Converting a polymeric carrier molecule, such as a polyol, into a chloroformate with phosgene gives rise to carbamate linkages to lysin groups in the polypeptide chain. This theme can be played in many variants substituting the chlorine with N-hydroxy succinimide, imidazole, para-nitrophenol, DMAP. The derivatives are usually made by reacting the chloroformate with the  
15 desired leaving group. All these groups give rise to carbamate linkages to the polypeptide.

Furthermore, isocyanates and isothiocyanates may be employed  
20 yielding ureas and thioureas, respectively.

Amides may be obtained from polyol acids using the same leaving groups as mentioned above and cyclic imid thrones.

Polyol succinate made from reaction with succinic anhydride can  
25 also be used. The hereby comprised ester group make the conjugate much more susceptible to hydrolysis. This group may be activated with N-hydroxy succinimide.

Furthermore, a special linker can be introduced. The oldest  
30 being cyanuric chloride (Abuchowski et al., (1977), J. Biol. Chem., 252, 3578-3581; Shafer et al., (1986), J. Polym. Sci. Polym. Chem. Ed., 24, 375-378.

Coupling to the polymeric carrier molecule of aromatic amine  
35 followed by diazotation yields a very reactive diazonium salt which *in situ* can be reacted with polypeptide molecules. An amide linkage may also be obtained by reacting an azlactone

derivative of polyols thus introducing an additional amide linkage.

5 Amino-groups of the polypeptide molecules may also be attached to polyols with carbamate linkages. Lysine residues may be used as the backbone.

In a specific embodiment two or more polypeptide molecules are coupled to each polymeric carrier molecules by

- 10 a) activating said polymeric carrier molecule by coupling thereto a reactive moiety, and  
b) reacting two or more polypeptide molecules with said activated polymeric carrier molecule.

- 15 In an embodiment of the invention the activation in step a) is performed by covalently linking thereto a reactive moiety derived from divinyl sulfone.

- 20 Details on how to conjugate one or more polypeptide molecules to each polymeric carrier molecule using divinyl sulfone as the attachment group is provided in for instance WO 93/01498 from Immunodex K/S, Denmark.

#### Compositions

- 25 The invention also relates to compositions comprising a polypeptide conjugate of the invention.

- Said compositions may further comprise polypeptides, such as proteins and/or enzymes and/or ingredients normally used in  
30 e.g. detergents, including soap bars, household articles, agrochemicals, personal care products, such as cleaning preparations e.g. for contact lenses, cosmetics, toiletries, oral and dermal pharmaceuticals, composition use for treating textiles, compositions for cleaning hard surfaces, compositions used for  
35 manufacturing food, e.g. baking, and feed etc.

Examples of polypeptides being enzymes include proteases, li-

pases, oxidoreductases, carbohydrases, transferases, such as transglutaminases, anti-microbial polypeptides, and phytases.

#### Detergent Compositions

5 Polypeptide conjugates of the invention, such as enzyme conjugates, may typically be a component of a detergent composition, e.g., a laundry detergent composition or a dishwashing detergent composition. As such, it may be included in the detergent composition in the form of a non-dusting granulate, a stabilized  
10 liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG)  
15 with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and  
20 triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid  
25 according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any  
30 convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants,  
35 each of which may be anionic, non-ionic, cationic, or amphoteric (zwitterionic). The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzenesulfonate

(LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also  
5 contain 0-40% of non-ionic surfactant such as alcohol ethoxylate (AEO or AE), alcohol propoxylate, carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyl dimethylamine oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty  
10 acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more enzymes, such as amylases, pullulanase, esterase, lipase, cutinase, protease, cellulase, peroxidase, or oxidase, e.g., lac-  
15 case, and anti-microbial polypeptides. One, more or all these polypeptides such as enzymes may be modified (i.e. conjugated) according to the invention.

Normally the detergent contains 1-65% of a detergent builder,  
20 but some dishwashing detergents may contain even up to 90% of a detergent builder, or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g.  
25 SKS-6 from Hoechst).

The detergent builders may be subdivided into phosphorus-containing and non-phosphorous-containing types. Examples of  
30 phosphorus-containing inorganic alkaline detergent builders include the water-soluble salts, especially alkali metal pyrophosphates, orthophosphates, polyphosphates and phosphonates. Examples of non-phosphorus-containing inorganic builders include water-soluble alkali metal carbonates, borates and  
35 silicates as well as layered disilicates and the various types of water-insoluble crystalline or amorphous aluminosilicates of which zeolites is the best known representative.

Examples of suitable organic builders include alkali metal, ammonium or substituted ammonium salts of succinates, malonates, fatty acid malonates, fatty acid sulphonates, carboxymethoxy succinates, polyacetates, carboxylates, polycarboxylates, aminopolycarboxylates and polyacetyl carboxylates.

The detergent may also be unbuilt, i.e. essentially free of detergent builder.

10

The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, polymaleates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

15

The detergent composition may contain bleaching agents of the chlorine/bromine-type or the oxygen-type. The bleaching agents may be coated or encapsulated. Examples of inorganic chlorine/bromine-type bleaches are lithium, sodium or calcium hypochlorite or hypobromite as well as chlorinated trisodium phosphate. The bleaching system may also comprise a  $H_2O_2$  source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylene-diamine (TAED) or nonanoyloxybenzenesulfonate (NOBS).

20

25

Examples of organic chlorine/bromine-type bleaches are heterocyclic N-bromo and N-chloro imides such as trichloroisocyanuric, tribromoisocyanuric, dibromoisocyanuric and dichloroisocyanuric acids, and salts thereof with water solubilizing cations such as potassium and sodium. Hydantoin compounds are also suitable. The bleaching system may also comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

30

In dishwashing detergents the oxygen bleaches are preferred, for example in the form of an inorganic persalt, preferably with a bleach precursor or as a peroxy acid compound. Typical

35

examples of suitable peroxy bleach compounds are alkali metal perborates, both tetrahydrates and monohydrates, alkali metal percarbonates, persilicates and perphosphates. Preferred activator materials are TAED or NOBS.

5

The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative  
10 such as, e.g., an aromatic borate ester, and the composition may be formulated as described in, e.g., WO 92/19709 and WO 92/19708. The enzymes of the invention may also be stabilized by adding reversible enzyme inhibitors, e.g., of the protein type as described in EP 0 544 777 B1.

15

The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, deflocculant material, foam boosters/foam depressors (in dishwashing detergents foam depressors), suds suppressors,  
20 anti-corrosion agents, soil-suspending agents, anti-soil-redeposition agents, dyes, dehydrating agents, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will  
25 usually be neutral or alkaline, e.g. in the range of 7-11.

Particular forms of laundry detergent compositions within the scope of the invention include:

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	7 - 12%
Alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO) or alkyl sulfate (e.g. C <sub>16-18</sub> )	1 - 4%
Alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14 - 20%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	2 - 6%
Zeolite (as NaAlSiO <sub>4</sub> )	15 - 22%
Sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0 - 6%
Sodium citrate/citric acid (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )	0 - 15%
Sodium perborate (as NaBO <sub>3</sub> ·H <sub>2</sub> O)	11 - 18%
TAED	2 - 6%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001 - 0.5%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener, photobleach)	0 - 5%



2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	6 - 11%
Alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO or alkyl sulfate (e.g. C <sub>16-18</sub> ))	1 - 3%
Alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	15 - 21%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	1 - 4%
Zeolite (as NaAlSiO <sub>4</sub> )	24 - 34%
Sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	4 - 10%
Sodium citrate/citric acid (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )	0 - 15%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 6%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001 - 0.5%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	5	- 9%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO)	7	- 14%
Soap as fatty acid (e.g. C <sub>16-22</sub> fatty acid)	1	- 3%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	10	- 17%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	3	- 9%
Zeolite (as NaAlSiO <sub>4</sub> )	23	- 33%
Sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	- 4%
Sodium perborate (as NaBO <sub>3</sub> ·H <sub>2</sub> O)	8	- 16%
TAED	2	- 8%
Phosphonate (e.g. EDTMPA)	0	- 1%
Carboxymethylcellulose	0	- 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0	- 3%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener)	0	- 5%

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	8	- 12%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO)	10	- 25%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14	- 22%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	1	- 5%
Zeolite (as NaAlSiO <sub>4</sub> )	25	- 35%
Sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	- 10%
Carboxymethylcellulose	0	- 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1	- 3%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. suds suppressors, perfume)	0	- 5%

## 5) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15	- 21%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO or C <sub>12-15</sub> alcohol, 5 EO)	12	- 18%
Soap as fatty acid (e.g. oleic acid)	3	- 13%
Alkenylsuccinic acid (C <sub>12-14</sub> )	0	- 13%
Aminoethanol	8	- 18%
Citric acid	2	- 8%
Phosphonate	0	- 3%
Polymers (e.g. PVP, PEG)	0	- 3%
Borate (as B <sub>4</sub> O <sub>7</sub> )	0	- 2%
Ethanol	0	- 3%
Propylene glycol	8	- 14%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. dispersants, suds suppressors, perfume, optical brightener)	0	- 5%

6) An aqueous structured liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15	- 21%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO, or C <sub>12-15</sub> alcohol, 5 EO)	3	- 9%
Soap as fatty acid (e.g. oleic acid)	3	- 10%
Zeolite (as NaAlSiO <sub>4</sub> )	14	- 22%
Potassium citrate	9	- 18%
Borate (as B <sub>4</sub> O <sub>7</sub> )	0	- 2%
Carboxymethylcellulose	0	- 2%
Polymers (e.g. PEG, PVP)	0	- 3%
Anchoring polymers such as, e.g., lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1; MW 3800	0	- 3%
Glycerol	0	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. dispersants, suds suppressors, per- fume, optical brighteners)	0	- 5%

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Fatty alcohol sulfate	5	- 10%
Ethoxylated fatty acid monoethanol- amide	3	- 9%
Soap as fatty acid	0	- 3%
Sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	5	- 10%
Soluble silicate (as $\text{Na}_2\text{O}, 2\text{SiO}_2$ )	1	- 4%
Zeolite (as $\text{NaAlSiO}_4$ )	20	- 40%
Sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	2	- 8%
Sodium perborate (as $\text{NaBO}_3 \cdot \text{H}_2\text{O}$ )	12	- 18%
TAED	2	- 7%
Polymers (e.g. maleic/acrylic acid copolymer, PEG)	1	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. optical brightener, suds suppressors, per- fume)	0	- 5%

8) A detergent composition formulated as a granulate comprising

Linear alkylbenzenesulfonate (calculated as acid)	8	- 14%
Ethoxylated fatty acid monoethanol- amide	5	- 11%
Soap as fatty acid	0	- 3%
Sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	4	- 10%
Soluble silicate (as $\text{Na}_2\text{O}, 2\text{SiO}_2$ )	1	- 4%
Zeolite (as $\text{NaAlSiO}_4$ )	30	- 50%
Sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	3	- 11%
Sodium citrate (as $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ )	5	- 12%
Polymers (e.g. PVP, maleic/acrylic acid copolymer, PEG)	1	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. suds suppressors, perfume)	0	- 5%

9) A detergent composition formulated as a granulate comprising

Linear alkylbenzenesulfonate (calculated as acid)	6	- 12%
Nonionic surfactant	1	- 4%
Soap as fatty acid	2	- 6%
Sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	14	- 22%
Zeolite (as $\text{NaAlSiO}_4$ )	18	- 32%
Sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	5	- 20%
Sodium citrate (as $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ )	3	- 8%
Sodium perborate (as $\text{NaBO}_3 \cdot \text{H}_2\text{O}$ )	4	- 9%
Bleach activator (e.g. NOBS or TAED)	1	- 5%
Carboxymethylcellulose	0	- 2%
Polymers (e.g. polycarboxylate or PEG)	1	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. optical brightener, perfume)	0	- 5%



10) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15	- 23%
Alcohol ethoxysulfate (e.g. C <sub>12-15</sub> alcohol, 2-3 EO)	8	- 15%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> al- cohol, 7 EO, or C <sub>12-15</sub> alcohol, 5 EO)	3	- 9%
Soap as fatty acid (e.g. lauric acid)	0	- 3%
Aminoethanol	1	- 5%
Sodium citrate	5	- 10%
Hydrotrope (e.g. sodium toluensulfonate)	2	- 6%
Borate (as B <sub>4</sub> O <sub>7</sub> )	0	- 2%
Carboxymethylcellulose	0	- 1%
Ethanol	1	- 3%
Propylene glycol	2	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. polymers, dispersants, perfume, optical brighteners)	0	- 5%

11) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	20	- 32%
Alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO, or C <sub>12-15</sub> alcohol, 5 EO)	6	- 12%
Aminoethanol	2	- 6%
Citric acid	8	- 14%
Borate (as B <sub>4</sub> O <sub>7</sub> )	1	- 3%
Polymer (e.g. maleic/acrylic acid copolymer, anchoring polymer such as, e.g., lauryl methacrylate/acrylic acid copolymer)	0	- 3%
Glycerol	3	- 8%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. hydrotropes, dispersants, perfume, optical brighteners)	0	- 5%

12). A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Anionic surfactant (linear alkylbenzenesulfonate, alkyl sulfate, alpha-olefinsulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap)	25	- 40%
Nonionic surfactant (e.g. alcohol ethoxylate)	1	- 10%
Sodium carbonate (as $\text{Na}_2\text{CO}_3$ )	8	- 25%
Soluble silicates (as $\text{Na}_2\text{O}$ , $2\text{SiO}_2$ )	5	- 15%
Sodium sulfate (as $\text{Na}_2\text{SO}_4$ )	0	- 5%
Zeolite (as $\text{NaAlSiO}_4$ )	15	- 28%
Sodium perborate (as $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ )	0	- 20%
Bleach activator (TAED or NOBS)	0	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. perfume, optical brighteners)	0	- 3%

13) Detergent formulations as described in 1) - 12) wherein all or part of the linear alkylbenzenesulfonate are replaced by (C<sub>12</sub>-C<sub>18</sub>) alkyl sulfate.

- 5 14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

(C <sub>12</sub> -C <sub>18</sub> ) alkyl sulfate	9	- 15%
Alcohol ethoxylate	3	- 6%
Polyhydroxy alkyl fatty acid amide	1	- 5%
Zeolite (as NaAlSiO <sub>4</sub> )	10	- 20%
Layered disilicate (e.g. SK56 from Hoechst)	10	- 20%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	3	- 12%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	0	- 6%
Sodium citrate	4	- 8%
Sodium percarbonate	13	- 22%
TAED	3	- 8%
Polymers (e.g. polycarboxylates and PVP)	0	- 5%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. optical brightener, photo bleach, perfume, suds suppressors)	0	- 5%

15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

(C <sub>12</sub> -C <sub>18</sub> ) alkyl sulfate	4	- 8%
Alcohol ethoxylate	11	- 15%
Soap	1	- 4%
Zeolite MAP or zeolite A	35	- 45%
Sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	2	- 8%
Soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	0	- 4%
Sodium percarbonate	13	- 22%
TAED	1	- 8%
Carboxymethyl cellulose	0	- 3%
Polymers (e.g. polycarboxylates and PVP)	0	- 3%
Enzymes including modified enzymes (calculated as pure enzyme protein)	0.0001	- 0.5%
Minor ingredients (e.g. optical brightener, phosphonate, perfume)	0	- 3%

- 5 16) Detergent formulations as described in 1) - 15) which contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for already specified bleach systems.
- 10 17) Detergent compositions as described in 1), 3), 7), 9) and 12) wherein perborate is replaced by percarbonate.
- 18) Detergent compositions as described in 1), 3), 7), 9), 12), 14) and 15) which additionally contain a manganese catalyst.
- 15 The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature, 369, (1994), p. 637-639.
- 19) Detergent composition formulated as a nonaqueous detergent
- 20 liquid comprising a liquid nonionic surfactant such as, e.g., linear alkoxyated primary alcohol, a builder system (e.g.

phosphate), enzyme and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

Particular forms of dishwashing detergent compositions within  
5 the scope of the invention include:

1) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant	0.4	- 2.5%
Sodium metasilicate	0	- 20%
Sodium disilicate	3	- 20%
Sodium triphosphate	20	- 40%
Sodium carbonate	0	- 20%
Sodium perborate	2	- 9%
Tetraacetythylenediamine (TAED)	1	- 4%
Sodium sulphate	5	- 33%
Enzymes including modified enzymes	0.0001	- 0.5%

## 2) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant (e.g. alcohol ethoxylate)	1	- 2%
Sodium disilicate	2	- 30%
Sodium carbonate	10	- 50%
Sodium phosphonate	0	- 5%
Trisodium citrate dihydrate	9	- 30%
Nitrilotrisodium acetate (NTA)	0	- 20%
Sodium perborate monohydrate	5	- 10%
Tetraacetylenediamine (TAED)	1	- 2%
Polyacrylate polymer (e.g. maleic acid/acrylic acid co-polymer)	6	- 25%
Enzymes including modified enzymes	0.0001	- 0.5%
Perfume	0.1	- 0.5%
Water	5	- 10

5

## 3) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant	0.5	- 2.0%
Sodium disilicate	25	- 40%
Sodium citrate	30	- 55%
Sodium carbonate	0	- 29%
Sodium bicarbonate	0	- 20%
Sodium perborate monohydrate	0	- 15%
Tetraacetylenediamine (TAED)	0	- 6%
Maleic acid/acrylic acid copolymer	0	- 5%
Clay	1	- 3%
Poly(amino acids)	0	- 20%
Sodium polyacrylate	0	- 8%
Enzymes including modified enzymes	0.0001	- 0.5%

## 4) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant	1	- 2%
Zeolite MAP	15	- 42%
Sodium disilicate	30	- 34%
Sodium citrate	0	- 12%
Sodium carbonate	0	- 20%
Sodium perborate monohydrate	7	- 15%
Tetraacetylenediamine (TAED)	0	- 3%
Polymer	0	- 4%
Maleic acid/acrylic acid copolymer	0	- 5%
Organic phosphonate	0	- 4%
Clay	1	- 2%
Enzymes including modified enzymes	0.0001	- 0.5%
Sodium sulphate	Balance	

## 5) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant	1	- 7%
Sodium disilicate	18	- 30%
Trisodium citrate	10	- 24%
Sodium carbonate	12	- 20%
Monopersulphate (2 KHSO <sub>5</sub> .KHSO <sub>4</sub> .K <sub>2</sub> SO <sub>4</sub> )	15	- 21%
Bleach stabilizer	0.1	- 2%
Maleic acid/acrylic acid copolymer	0	- 6%
Diethylenetriaminepentaacetate, pentasodium salt	0	- 2.5%
Enzymes including modified enzymes	0.0001	- 0.5%
Sodium sulphate, water	Balance	



6) POWDER AND LIQUID DISHWASHING COMPOSITION WITH CLEANING SURFACTANT SYSTEM

Nonionic surfactant	0	-	1.5%
Octadecyl dimethylamine N-oxide dihydrate	0	-	5%
80:20 wt.C18/C16 blend of octadecyl dimethylamine N-oxide dihydrate and hexadecyldimethyl amine N-oxide dihydrate	0	-	4%
70:30 wt.C18/C16 blend of octadecyl bis (hydroxyethyl)amine N-oxide anhydrous and hexadecyl bis (hydroxyethyl)amine N-oxide anhydrous	0	-	5%
C <sub>13</sub> -C <sub>15</sub> alkyl ethoxysulfate with an average degree of ethoxylation of 3	0	-	10%
C <sub>12</sub> -C <sub>15</sub> alkyl ethoxysulfate with an average degree of ethoxylation of 3	0	-	5%
C <sub>13</sub> -C <sub>15</sub> ethoxylated alcohol with an average degree of ethoxylation of 12	0	-	5%
A blend of C <sub>12</sub> -C <sub>15</sub> ethoxylated alcohols with an average degree of ethoxylation of 9	0	-	6.5%
A blend of C <sub>13</sub> -C <sub>15</sub> ethoxylated alcohols with an average degree of ethoxylation of 30	0	-	4%
Sodium disilicate	0	-	33%
Sodium tripolyphosphate	0	-	46%
Sodium citrate	0	-	28%
Citric acid	0	-	29%
Sodium carbonate	0	-	20%
Sodium perborate monohydrate	0	-	11.5%
Tetraacetythylenediamine (TAED)	0	-	4%
Maleic acid/acrylic acid copolymer	0	-	7.5%
Sodium sulphate	0	-	12.5%
Enzymes including modified enzymes	0.0001	-	0.5%

## 7) NON-AQUEOUS LIQUID AUTOMATIC DISHWASHING COMPOSITION

Liquid nonionic surfactant (e.g. alcohol ethoxylates)	2.0	- 10.0%
Alkali metal silicate	3.0	- 15.0%
Alkali metal phosphate	20.0	- 40.0%
Liquid carrier selected from higher glycols, polyglycols, polyoxides, glycolethers	25.0	- 45.0%
Stabilizer (e.g. a partial ester of phosphoric acid and a C <sub>16</sub> -C <sub>18</sub> alkanol)	0.5	- 7.0%
Foam suppressor (e.g. silicone)	0	- 1.5%
Enzymes including modified enzymes	0.0001	- 0.5%

## 5 8) NON-AQUEOUS LIQUID DISHWASHING COMPOSITION

Liquid nonionic surfactant (e.g. alcohol ethoxylates)	2.0	- 10.0%
Sodium silicate	3.0	- 15.0%
Alkali metal carbonate	7.0	- 20.0%
Sodium citrate	0.0	- 1.5%
Stabilizing system (e.g. mixtures of finely divided silicone and low molecular weight dialkyl polyglycol ethers)	0.5	- 7.0%
Low molecule weight polyacrylate polymer	5.0	- 15.0%
Clay gel thickener (e.g. bentonite)	0.0	- 10.0%
Hydroxypropyl cellulose polymer	0.0	- 0.6%
Enzymes including modified enzymes	0.0001	- 0.5%
Liquid carrier selected from higher glycols, polyglycols, polyoxides and glycol ethers	Balance	

## 9) THIXOTROPIC LIQUID AUTOMATIC DISHWASHING COMPOSITION

C <sub>12</sub> -C <sub>14</sub> fatty acid	0	- 0.5%
Block co-polymer surfactant	1.5	- 15.0%
Sodium citrate	0	- 12%
Sodium tripolyphosphate	0	- 15%
Sodium carbonate	0	- 8%
Aluminium tristearate	0	- 0.1%
Sodium cumene sulphonate	0	- 1.7%
Polyacrylate thickener	1.32	- 2.5%
Sodium polyacrylate	2.4	- 6.0%
Boric acid	0	- 4.0%
Sodium formate	0	- 0.45%
Calcium formate	0	- 0.2%
Sodium n-decydiphenyl oxide disulphonate	0	- 4.0%
Monoethanol amine (MEA)	0	- 1.86%
Sodium hydroxide (50%)	1.9	- 9.3%
1,2-Propanediol	0	- 9.4%
Enzymes including modified enzymes	0.0001	- 0.5%
Suds suppressor, dye, perfumes, water	Balance	

## 10) LIQUID AUTOMATIC DISHWASHING COMPOSITION

Alcohol ethoxylate	0	- 20%
Fatty acid ester sulphonate	0	- 30%
Sodium dodecyl sulphate	0	- 20%
Alkyl polyglycoside	0	- 21%
Oleic acid	0	- 10%
Sodium disilicate monohydrate	18	- 33%
Sodium citrate dihydrate	18	- 33%
Sodium stearate	0	- 2.5%
Sodium perborate monohydrate	0	- 13%
Tetraacetylenediamine (TAED)	0	- 8%
Maleic acid/acrylic acid copolymer	4	- 8%
Enzymes including modified enzymes	0.0001	- 0.5%

## 5 11) LIQUID AUTOMATIC DISHWASHING COMPOSITION CONTAINING PROTECTED BLEACH PARTICLES

Sodium silicate	5	- 10%
Tetrapotassium pyrophosphate	15	- 25%
Sodium triphosphate	0	- 2%
Potassium carbonate	4	- 8%
Protected bleach particles, e.g. chlorine	5	- 10%
Polymeric thickener	0.7	- 1.5%
Potassium hydroxide	0	- 2%
Enzymes including modified enzymes	0.0001	- 0.5%
Water	Balance	

11) Automatic dishwashing compositions as described in 1), 2),  
 10 3), 4), 6) and 10), wherein perborate is replaced by percarbonate.

12) Automatic dishwashing compositions as described in 1) - 6)  
 which additionally contain a manganese catalyst. The manganese  
 15 catalyst may, e.g., be one of the compounds described in "Effi-

cient manganese catalysts for low-temperature bleaching", Nature, 369, (1994), p. 637-639.

#### **Personal care applications**

- 5 Also for personal care products enzyme conjugates with reduced allergenicity of the invention are of interest.

#### **Proteases**

- 10 Proteases are well-known active ingredients for cleaning of contact lenses. They hydrolyse the proteinaceous soil on the lens and thereby makes it soluble. Removal of the protein soil is essential for the wearing comfort.

- 15 Proteases are also effective ingredients in skin cleaning products, where they remove the upper layer of dead keratinaceous skin cells and thereby make the skin look brighter and more fresh.

- 20 Proteases are also used in oral care products, especially for cleaning of dentures, but also in dentifrices.

- 25 Further, proteases are used in toiletries, bath and shower products, including shampoos, conditioners, lotions, creams, soap bars, toilet soaps, and liquid soaps.

#### **Lipases**

- 30 Lipases can be applied for cosmetic use as active ingredients in skin cleaning products and anti-acne products for removal of excessive skin lipids, and in bath and shower products such as creams and lotions as active ingredients for skin care.

- 35 Lipases can also be used in hair cleaning products (e.g. shampoos) for effective removal of sebum and other fatty material from the surface of hair.

- Lipases are also effective ingredients in products for cleaning of contact lenses, where they remove lipid deposits from the

lens surface.

### Oxidoreductases

The most common oxidoreductase for personal care purposes is an  
5 oxidase (usually glucose oxidase) with substrate (e.g. glucose)  
that ensures production of  $H_2O_2$ , which then will initiate the  
oxidation of for instance  $SCN^-$  or  $I^-$  into antimicrobial re-  
agents ( $SCNO^-$  or  $I_2$ ) by a peroxidase (usually lactoperoxidase).  
This enzymatic complex is known in nature from e.g. milk and  
10 saliva.

It is being utilised commercially as anti-microbial system in  
oral care products (mouth rinse, dentifrice, chewing gum) where  
it also can be combined with an amyloglucosidase to produce the  
15 glucose. These systems are also known in cosmetic products for  
preservation.

Anti-microbial systems comprising the combination of an oxidase  
and a peroxidase are known in the cleaning of contact lenses.  
20

Another application of oxidoreductases are oxidative hair dye-  
ing using oxidases, peroxidases and laccases.

Free radicals formed on the surface of the skin (and hair)  
25 known to be associated with the ageing process of the skin  
(spoilage of the hair).

The free radicals activate chain reactions that leads to  
destruction of fatty membranes, collagen, and cells.

30 The application of free radical scavengers such as Superoxide  
dismutase into cosmetics is well-known (R. L. Goldemberg, DCI,  
Nov. 93, p. 48-52).

Protein disulfide isomerase (PDI) is also an oxidoreductase. It  
35 can be utilised for waving of hair (reduction and reoxidation  
of disulfide bonds in hair) and repair of spoiled hair (where  
the damage is mainly reduction of existing disulfide bonds).

Carbohydrases

Plaque formed on the surface of teeth is composed mainly of polysaccharides. They stick to the surface of the teeth and the

5 microorganisms. The polysaccharides are mainly  $\alpha$ -1,6 bound glucose (dextran) and  $\alpha$ -1,3 bound glucose (mutan). The application of different types of glucanases such as mutanase and dextranase helps hydrolysing the sticky matrix of plaque, making it easier to remove by mechanical action.

10

Also other kinds of biofilm for instance the biofilm formed in lens cases can be removed by the action of glucanases.

Anti-microbial polypeptides

15 Anti-microbial polypeptides have widespread applications such as for preservation of cosmetic products, anti-acne products, deodorants and shampoos. Further such polypeptides may be use in contact lens products.

20 Specific product formulations

Examples of specific personal care compositions can be found in "Cosmetics and Toiletries" edited by Wilfried Umbach and published by Ellis Horwood, Limited, England, (1991), and "Surfactants in Consumer Products", edited by J. Falbe and

25 published by Spring-Verlag, (1987).

In the following a non exhausting list of guide formulations are listed. These provide an overview of formulations of important personal care products contemplated according to the invention.

30

**Toilet soap**

Ingredients	Examples	%
Surfactants	Soap (sodium salt)	83 -87
Sequestering agents	Ethylenediamine tetraacetate	0.1-0.3
35 Consistency regulators	Sodium chloride	approx.
	0.5	

	Dyestuffs		< 0.1
	Optical brighteners		< 0.1
	Antioxidants	2,6-bis(1,1-Dimethylethyl)- 4-methyl phenol(BHT)	0.1-0.3
5	Whitening agents	Titanium dioxide	0.1-0.3
	Fragrances		1.0-2.0
	Enzymes	Protease/Lipase	0-5
	Water		Balance
10	<b>Syndet (Synthetic Detergents)</b>		
	Ingredients	Examples	%
	Surfactants	Lauryl sulfate	30-50
		Lauryl sulfo succinate	1-12
	Refatting agents	Fatty alcohols	10-20
15	Plasticizers	Stearyl mono/diglycerides	0-10
	Fillers	Starches	0-10
	Active agents	Salicylic acid	0-1
	Dyestuffs		< 0.2
	Fragrances		0-2
20	Enzymes	Protease/Lipase	0-5
	Water		Balance
	<b>Foam bath and shower bath</b>		
	Ingredients	Examples	%
25			Foam bath
	Surfactants	Lauryl ether sulfate	10-20
		Coco amidopropyl dimethyl betaine	2-4
30		Ethoxylated fatty acids	0.5-2
	Refatting agents	Fatty alcohols	0.5-3
		Ethoxylated fatty alcohols	0.5-5
	Enzymes	Protease/Lipase	0-5
35			0-5



	Ingredients	Examples	%	
			Foam bath	Shower bath
5	Foam stabilizers	Fatty acid alkanol amides	0.2-2	0-4
	Conditioners	Quaternized hydroxypropyl cellulose	-	0-0.5
	Thickeners	Sodium chloride	0-3	0-3
	Pearlescent agents	Ethyleneglycol stearate	0-2	-
10	Active agents	Vegetable extracts	0-1	0-1
	Preservatives	5-Bromo-5-nitro-1,3-dioxane	0.1	0.1
	Dyestuffs		0.1-0.2	0.1
	Fragrances		0.3-3	0.3-2
15	Enzymes	Protease/Lipase	0-5	0-5
	Water		Balance	Balance

**Skin cream (water-in-oil type and oil-in-water type)**

	Ingredients	Examples	%	
			Water-in-oil/ Oil-in-water type	type
25	Emulsifiers	Sorbitane sesquioleate	3-5	-
		Aluminum stearate	1-2	-
		Triethanolamine stearate	-	1-2
		Cetyl/Stearyl alcohol polyglycol ethers	-	1-3
30	Fatty derivatives	Isopropyl palmitate	1-5	0-3
		Cetyl/Stearyl alcohol	-	0-2
		2-Octyl dodecanol	2-10	3-7
		Stearic/Palmitic acid	-	0-3
		Caprylic/Capric acid triglycerides	5-10	-
35	Moisturizers	Glycerine stearate	-	0-5
		Glycerine	1-5	1-5

		Sorbitol	1-5	1-5
		Poly (hydroxy carboxylic acids)	0.5-2	-
		Propyleneglycol	-	0-3
5	Stabilizers	Magnesium sulfate	0-0.8	-
	Preservatives	p-Hydroxy benzoic acid ester	0.2- 0.4	0.2-0.4
	Enzymes	Protease/Lipase	0-5	0-5
	Water	Balance	Balance	Balance
10				

**Body lotion (oil-in-water type) and skin lotion for application on the wet skin**

	Ingredients	Examples	% Body lotion	% Skin lotion
15				
	Emulsifiers	Cetyl/Stearyl alcohol polyglycol ethers	1 -3	-
		Sorbitane monolaurate	0.5-1	-
20		Sodium stearate	-	1-2
		Sodium lauryl ether sulfate	-	0.5-2
	Fatty derivatives	2-Octyl dodecanol	1-3	0-5
		Paraflin oils	-	20-25
25		Bees wax	0.5-1	-
		Isooctyl stearate	3-7	-
		Isopropyl palmitate	-	2-5
	Moisturizers	Glycerine	3-5	5-10
		Sorbitol	-	0-5
30	Thickeners	Polyacrylates	0-0.3	0-1
		Methyl hydroxypropyl cellulose	0-0.3	0-0.5
	Preservatives	p-Hydroxy benzoic acid ester	0.2-0.4	0.2-0.4
35	Enzymes	Protease/Lipase	0-5	0-5
	Water	Balance	Balance	Balance

<b>Face lotion</b>			
	<b>Ingredients</b>	<b>Examples</b>	<b>%</b>
5	Surfactants	Magnesium lauryl ether sulfate	0.2-0.5
	Refatting agents	Di-n-butyl adipate	1-2
	Solubilizers	Castor oil polyglycol ethers	0.1-1
	Cleaning and refreshing components	Ethanol	0-15
10	Moisturizers	Glycerine	0-5
		Sorbitol	0-5
	Preservatives	p-Hydroxy benzoic acid ester	0.2-0.4
15	Adstringents	Vegetable extracts	1-5
	Antiiirritants	Panthenol	0-1
		Allantoine	0-0.2
		Vegetable extracts	0.5-3
20	Enzymes	Protease/Lipase	0-5
	Water		Balance
<b>Hair shampoo</b>			
	<b>Ingredients</b>	<b>Examples</b>	<b>%</b>
25	Surfactants	Lauryl ether sulfate	12-16
		Coco fatty acid amidopropyl dimethyl betaine	2-5
		Fatty acid polyglycol esters	0-2
	Foam boosters	Fatty acid ethanol amides	0.5-2.5
30	Conditioners	Quaternized hydroxyethyl cellulose	0.4-1
		Protein hydrolysates	0.2-1
	Refatting agents	Ethoxylated lanolin alcohols	0.2-1
	Additives	Anti-dandruff agents	0-1
35	Preservatives	5-Bromo-5-nitro-1,3-dioxane	0.1-0.3
	Pearlescent agents	Ethyleneglycol stearate	0-2

	Dyestuffs		< 0.1
	pH-Regulators	Acids/Bases	0.1-1
	Fragrances		0.3-0.5
	Enzymes	Protease/Lipase	0-5
5	Water		Balance

**Hair rinse and hair conditioner**

	Ingredients	Examples	% Hair rinse	% Hair
10		conditioner		
	Surfactants	Fatty alcohol poly- glycol ethers	0.1-0.2	1.5-2.5
15		Cetyl trimethyl ammonium chloride	0.5-1	-
		Dimethyl benzyl stearyl ammonium chloride	-	0.5-1
20	Refatting agents	Cetyl/Stearyl mono/ diglyceride	0.5-1.5	1.5-2.5
	Consistency regulators	Fatty alcohols	1-2.5	2.5-3.5
	Thickeners	Methyl hydroxypropyl cellulose	0.3-0.6	0.4-0.8
25	Conditioners	Quaternized hydroxyethyl cellulose	0.1-0.3	0.3-0.4
	Preservatives	p-Hydroxy benzoic acid ester	0.1-0.3	0.1-0.3
	Dyestuffs		<0.1	<0.1
30	pH-Regulators	Acids/Bases	0.1-1	0.1-1
	Fragrances		0.2-0.5	0.2-0.5
	Enzymes	Protease/Lipase	0-5	0-5
	Water		Balance	Balance

**Hair dyes**

	Ingredients	Examples	%
	Component 1:	Alkaline dyeing cream	
	Surfactants	Lauryl ether sulfate	1-4
5		Ethoxylated castor oil	1-2
	Consistency	Fatty alcohols	8-10
		regulators	
	Reductants	Sodium sulfite	0.8-1.2
	Buffers	Ammonium chloride	0.5-1
10	Sequestrants	1-Hydroxyethane-1,1-diphosphonic acid	0.1-0.2
	Alkaline agents	Ammonia	1.2-2
	Oxidation dyestuffs	Developing agents	1
		Coupling agents	1
15	Enzyme	Laccase	0-5
	Water		Balance
	Component II:	Hydrogen peroxide dispersion	
	Surfactants	Lauryl ether sulfate	0.5-1
20	Oxidants	Hydrogen peroxide	6-9
	Stabilizers	1-Hydroxyethane-1,1-diphosphonic acid	1-1.5
	Thickeners	Polyacrylates	3-5
	Enzyme	Laccase	0-5
25	Water		Balance

**Shaving cream**

	Ingredients	Examples	%
	Soaps	Palmitic/Stearic acid	30-40
30		Potassium hydroxide	5-7
		Sodium hydroxide	1-2
	Fatty components	Coconut oil	5-10
		Polyethyleneglycol	0-2
	Stabilizers	Sodium tetraborate	0-0.5
35		Sodium silicate	0-0.5
		Sorbitol	0-3

	Enzyme	Protease	0-5
	Water		Balance
	<b>Shaving lotion</b>		
5	Ingredients	Examples	%
	Disinfecting and phonic acid	Ethanol	40-80
	Refatting agents	Di-n-butyl adipate	1-2
	Solubilizers	Ethoxylated castor oil	0.5-1
10	Adstringents	Vegetable extracts	1-10
	Antirritants	Panthenol	0-0.5
		Vegetable extracts	0-2
	Stabilizers	Glycerine	0-5
		Sorbitol	0-5
15		Propyleneglycol	0-3
	Enzymes	Protease	0-5
	Water		Balance
	<b>Hair pomade</b>		
20	Ingredients	Examples	%
	Consistency regulators	Fatty alcohols	4-5
		Ethoxylated lanolin alcohols	3-6
	Mineral fats	Vaseline	45-52
25		Branched chain paraffins	10-18
	Antioxidants	2,6-bis(1,1-Dimethylethyl)- 4-methyl phenol (BHT)	0.5- 1
	Fragrances		0.2-0.4
	Dyestuffs		0.1
30	Enzymes	Lipase	0-5
	Emollients	Glycerine	Balance
	<b>Setting lotion</b>		
	Ingredients	Examples	%
35	Solvents	Isopropanol	12-20
	Film forming	Vinyl pyrrolidone/vinyl	

	components	acetate copolymers	2-3.5
	Softening agents	Vinyl pyrrolidone/dimethyl amino ethyl methacrylate	0.2-1
	Conditioners	Protein hydrolysates	0.2-0.5
5	Antistatics	Cetyl trimethyl ammonium chloride	0.1-0.5
	Emulsifiers	Etboxylated castor oil	0.1-0.5
	Fragrances		0.1-0.2
	Dyestuffs		< 0.1
10	Enzymes	Lipase	0-5
	Water		Balance

#### Food and Feed

- Further conjugated enzymes or polypeptides with reduced allergenicity according to the invention may advantageously be used in the manufacturing of food and feed.

#### Proteases

- The gluten in wheat flour is the essential ingredient responsible for the ability of flour to be used in baked foodstuffs. Proteolytic enzymes are sometimes needed to modify the gluten phase of the dough, e.g. a hard wheat flour can be softened with a protease.

- Neutrase® is a commercially available neutral metallo protease that can be used to ensure a uniform dough quality and bread texture, and to improve flavour. The gluten proteins are degraded either moderately or more extensively to peptides, whereby close control is necessary in order to avoid excessive softening of the dough.

Proteases are also used for modifying milk protein.

- To coagulate casein in milk when producing cheese proteases such as rennet or chymosin may be used.

In the brewery industry proteases are used for brewing with

unmalted cereals and for controlling the nitrogen content.

In animal feed products proteases are used so to speak to expand the animals digestion system.

5

#### Lipases

The application of lipase in the baking industry is rather new. Addition of lipase results in improved dough properties and an improved breadmaking quality in terms of larger volume, improved crumb structure and whiter crumb colour. The observed effect can be explained by a mechanism where the lipase changes the interaction between gluten and some lipids fragment during dough mixing. This results in an improved gluten network.

15 The flavour development of blue roan cheeses (e.g. Danablu), certain Italian cheese types and other dairy products containing butter fat are dependent on the degradation of milk fat into free fatty acids. Lipases may be used for developing flavour in such products.

20

In the oil- and fat producing industry lipases are used e.g. to minimize the amount of undesirable side-products, to modify fats by interesterification, and to synthesis of esters.

#### 25 Oxidoreductases

Further oxidoreductases with reduced allergenicity according to the invention may advantageously be used in the manufacturing of food and feed.

30 Several oxidoreductases are used for baking, glucose oxidase, lipoxigenase, peroxidase, catalase and combinations hereof. Traditionally, bakers strengthen gluten by adding ascorbic acid and potassium bromate. Some oxidoreductases can be used to replace bromate in dough systems by oxidation of free sulfhydryl units in gluten proteins. Hereby disulphide linkages are formed  
35 resulting in stronger, more elastic doughs with greater resistance.



Gluzyme™ (Novo Nordisk A/S) is a glucose oxidase preparation with catalase activity that can be used to replace bromate. The dough strengthen is measured as greater resistance to mechanical shock, better oven spring and larger loaf volume.

#### Carbohydrases

Flour has varying content of amylases leading to differences in the baking quality. Addition of amylases can be necessary in order to standardize the flour. Amylases and pentosanases generally provide sugar for the yeast fermentation, improve the bread volume, retard retrogradation, and decrease the staling rate and stickiness that results from pentosan gums. Examples of carbohydrases are given below.

Certain maltogenic amylases can be used for prolonging the shelf life of bread for two or more days without causing gumminess in the product. Selectively modifies the gelatinized starch by cleaving from the non-reducing end of the starch molecules, low molecular weight sugars and dextrans. The starch is modified in such a way that retrogradation is less likely to occur. The produced low-molecular-weight sugars improve the baked goods water retention capacity without creating the intermediate-length dextrans that result in gumminess in the finished product. The enzyme is inactivated during bread baking, so it can be considered a processing aid which does not have to be declared on the label. The overdosing of Novamyl can almost be excluded.

The bread volume can be improved by fungal  $\alpha$ -amylases which further provide good and uniform structure of the bread crumb. Said  $\alpha$ -amylases are endoenzymes that produce maltose, dextrans and glucose. Cereal and some bacterial  $\alpha$ -amylases are inactivated at temperatures above the gelatinization temperature of starch, therefore when added to a wheat dough it results in a low bread volume and a sticky bread interior. Fungamyl has the

advantage of being thermolabile and is inactivated just below the gelatinization temperature.

5 Enzyme preparations containing a number of pentosanase and hemi-cellulase activities can improve the handling and stability of the dough, and improves the freshness, the crumb structure and the volume of the bread.

10 By hydrolysing the pentosans fraction in flour, it will lose a great deal of its water-binding capacity, and the water will then be available for starch and gluten. The gluten becomes more pliable and extensible, and the starch gelatinize more easily. Pentosanases can be used in combination with or as an alternative to emulsifiers.

15 Further carbohydrases are user for producing syrups from starch, which are widely used in soft drinks, sweets, meat products, dairy products, bread products, ice cream, baby food, jam etc.

20 The conversion of starch is normally carried out in three steps. First the starch is liquefied, by the use of  $\alpha$ -amylases. Maltodextrins, primary consisting of oligosaccharides and dextrins, are obtained.

25 The mixture is then treated with an amyloglucosidase for hydrolysing the oligosaccharides and dextrins into glucose. This way a sweeter product is obtained. If high maltose syrups are desired  $\beta$ -amylases alone or in combination with a pullulanase (de-branching enzyme) may be used.

30 The glucose mixture can be made even sweeter by isomerization to fructose. For this an immobilized glucose isomerase can be used.

35 In the sugar industry, it is common practice to speed up the break down of present starch in cane juices. Thereby the starch

content in the raw sugar is reduced and filtration at the refinery is facilitated.

Furthermore dextranases are used to break down dextran in raw  
5 sugar juices and syrups.

In the alcohol industry  $\alpha$ -amylases are advantageously being used for thinning of starch in distilling mashes.

10 In the brewing industry  $\alpha$ -amylases are used for adjunct liquefaction.

In the dairy industry  $\beta$ -galactosidases (lactase) are used when producing low lactose milk for persons suffering from lactose  
15 malabsorption.

When flavoured milk drinks are produced from lactase-treated milk, the addition of sugar can be reduced without reducing the sweetness of the product.

20 In the production of condensed milk, lactose crystallization can be avoided by lactase treatment, and the risk of thickening caused by casein coagulation in lactose crystals is thus reduced.

25 When producing ice cream made from lactase-treated milk (or whey) no lactose crystals will be formed and the defect, sandiness, will not occur.

30 Further, xylanases are known to be used within a number of food/feed industrial applications as described in WO 94/21785 (Novo Nordisk A/S).

$\alpha$ -amylases are used in the animal feed industry to be added to  
35 cereal-containing feed to improve the digestibility of starch.

Anti-microbial polypeptides

Certain bacteriolytic enzymes may be used e.g. to wash carcasses in the meat packing industry (see US patent no. 5,354,681 from Novo Industri A/S)

5

Transferases

Transglutaminases with reduced allergenicity according to the invention may advantageously be used in the manufacturing of food and feed.

10

Transglutaminases have the ability to crosslinking protein.

This property can be used for gelling of aqueous phases containing proteins. This may be used for producing spreads (DK patent application no. 1071/84 from Novo Nordisk A/S).

15

Transglutaminases are being used for improvement of baking quality of flour e.g. by modifying wheat flour to be used in the preparation of cakes with improved properties, such as improved taste, dent, mouth-feel and a higher volume (see JP 1-110147).

20

Further producing paste type food material e.g. used as fat substitution in foods as ice cream, toppings, frozen desserts, mayonnaise and low fat spreads (see WO 93/22930 from Novo Nordisk A/S).

25

Furthermore for preparation of gels for yoghurt, mousses, cheese, puddings, orange juice, from milk and milk-like products, and binding of chopped meat product, improvement of taste and texture of food proteins (see WO 94/21120 and WO 94/21129 from Novo Nordisk A/S).

30

Phytases

Phytases of the invention may advantageously be used in the manufacturing of food, such as breakfast cereal, cake, sweets, drink, bread or soup etc., and animal feed.

35

Phytases may be used either for exploiting the phosphorus bound in the phytate/phytic acid present in vegetable protein sources or for exploiting the nutritionally important minerals bound in phytic acid complexes.

Microbial phytase may be added to feedstuff of monogastric animals in order to avoid supplementing the feed with inorganic phosphorus (see US patent no. 3,297,548)

Further phytases may be used in soy processing. Soy bean meal may contain high levels of the anti-nutritional factor phytate which renders this protein source unsuitable for application in baby food and feed for fish, calves and other non-ruminants, since the phytate chelates essential minerals present therein (see EP 0 420 358).

Also for baking purposes phytases may be used. Bread with better quality can be prepared by baking divided pieces of a dough containing wheat flour etc. and phytase (see JP-0-3076529-A)

A high phytase activity koji mold are known to be used for producing refined sake (see JP-0-6070749-A).

## Textile applications

### Proteases

Proteases are used for degumming and sand-washing of silk.

### Lipases

Lipases are used for removing fatty matter containing hydrophobic esters (e.g. triglycerides) during the finishing of textiles (see e.g. WO 93/13256 from Novo Nordisk A/S).

### Oxidoreductases

In bleach clean-up of textiles catalases may serve to remove excess hydrogen peroxide.

#### Carbohydrases

- 5 Cellulolytic enzymes are widely used in the finishing of denim garments in order to provide a localized variation in the colour density of the fabric (Enzyme facilitated "stone wash").

10 Also cellulolytic enzymes find use in the bio-polishing process. Bio-Polishing is a specific treatment of the yarn surface which improves fabric quality with respect to handle and appearance without loss of fabric wettability. Bio-polishing may be obtained by applying the method described e.g. in WO 93/20278.

15 During the weaving of textiles, the threads are exposed to considerable mechanical strain. In order to prevent breaking, they are usually reinforced by coating (sizing) with a gelatinous substance (size). The most common sizing agent is starch in  
20 native or modified form. A uniform and durable finishing can thus be obtained only after removal of the size from the fabric, the so called desizing. Desizing of fabrics sized with a size containing starch or modified starch is preferably facilitated by use of amylolytic enzymes.

25

#### **Oral and dermal pharmaceuticals**

##### Proteases

- 30 Different combinations of highly purified proteases (e.g. Trypsin and Chymotrypsin) are used in pharmaceuticals to be taken orally, and dermal pharmaceuticals for combating e.g. inflammations, edemata and injuries.

35

#### **Leather production**

Transferase

Transglutaminase is known to be used for casein finishing of leather by acting as a hardening agent (see WO 94/13839 from Novo Nordisk).

5

**Hard surface cleaning**

Cleaning of hard surfaces e.g. in the food industry is often difficult, as equipment used for producing dairies, meat, sea food products, beverages etc. often have a complicated shape.

10 The use of surfactant compositions in the form gels and foams comprising enzymes have shown to facilitate and improve hard surface cleaning. Enzymes, which advantageously may be added in such surfactant compositions, are in particular proteases, lipases, amylases and cellulases.

15

Such hard surface cleaning compositions comprising enzymes may also advantageously be used in the transport sector, for instance for washing cars and for general vessel wash.

20 Finally the invention relates to the use of the conjugate of the invention or a composition of the invention in products comprising polypeptides.

First of all the conjugate or compositions of the invention can  
25 advantageously be used for personal care products, such as hair care and hair treatment products. This include products such as shampoo, balsam, hair conditioners, hair waving compositions, hair dyeing compositions, hair tonic, hair liquid, hair cream, shampoo, hair rinse, hair spray.

30

Further contemplated is oral care products such as dentifrice, mouth washes, chewing gum.

Also contemplated are skin care products and cosmetics, such as  
35 skin cream, skin milk, cleansing cream, cleansing lotion, cleansing milk, cold cream, cream soap, nourishing essence, skin lotion, milky lotion, calamine lotion, hand cream, powder

soap, transparent soap, sun oil, sun screen, shaving foam, shaving cream, baby oil lipstick, lip cream, creamy foundation, face powder, powder eye-shadow, powder, foundation, make-up base, essence powder, whitening powder.

5

Also for contact lenses hygiene products the conjugate of the invention can be used advantageously. Such products include contact lenses cleaning and disinfection products.

- 10 The use for detergents such as washing powder, soap, soap bars, liquid soap are also contemplated.

#### Materials and Method

15

##### Enzymes:

Monomer purified peroxidase ( $M_r = 39$  kDa) derived from wild-type *Coprinus cinereus* (available from Novo Nordisk A/S).

Lipase: Lipolase® (from Novo Nordisk A/S)

- 20 Cellulase: Carezyme® core prepared as described according to Boisset, C. et al. (1995), FEBS Lett. 376, p. 49-52.

Dextran-peroxidase A (prepared by Kem-En-Tech, Denmark).

Dextran-peroxidase B (prepared by Kem-En-Tech, Denmark).

Dextran-cellulase (prepared by Kem-En-Tech, Denmark).

- 25 Dextran-lipase (prepared by Kem-En-Tech, Denmark).

##### Solutions

PBS Tween20 Ausubel, F.M. et al. (Editors), 1994

Rabbit anti-goat (Sigma A-4187)

- 30 Alkaline phosphatase Buffer (pH=9.0)

NaCl 5.844 g

MgCl<sub>2</sub>·6H<sub>2</sub>O 1.02 g

Diethanol amine 10.51 g

- The pH is adjusted to 9.0 with HCl, and Milli-Q water is  
35 applied to 1 litre.

Stop-solution



EDTA, disodium 74.44 g

K<sub>2</sub>HPO<sub>4</sub> 174.2 g

NAH<sub>3</sub> 0.2 g

The pH is adjusted to 10 with about 22.5 g KOH in Milli-Q water  
5 to 1 litre.

#### Test animals

Dunkin Hartley guinea pigs (from Charles River, DE)

#### 10 Equipment

ELISA reader: Ceres 900 HDi

#### Preparation of anti-lipase and anti-cellulase, anti-peroxidase

White New Zealand rabbits are used. 2-4 rabbits per antigen.

15 FCI(Freund incomplete adjuvant) is used.

50-100 µg enzyme protein is used per rabbit per injection.

For mono-specific antibody production very high purified antigen is used.

20 Each rabbit is given an injection in the back of the neck (subcutaneous) with 1 ml fresh stable mix of 1:1 antigen and adjuvant.

Injection weekly up till 10 weeks.

25

1 blood sample from the rabbit is taken after the fifth immunization and checked in Ouchterlony (Nils H Axelsen, (1983), "Handbook of Immunoprecipitation-in-Gel Techniques", Blackwell Scientific Publication) when the titer shows 1/18-

30 1/32 blood is drawn from the rabbits and antibodies are purified.

#### ELISA procedure for determination of IgG<sub>1</sub> positive guinea pigs

ELISA microtiter plates are coated with rabbit anti-peroxidase

35 1:8000, rabbit anti-cellulase 1:8000 and anti-lipase 1:6000, respectively, in carbonate buffer and incubated over night at 4°C. The next day the plates are blocked with 2% BSA for 1 hour

and washed 3 times with PBS tween 20.

Peroxidase, cellulase Core and lipase were added to the relevant plates. 1µg enzyme protein/ml.

5

All guinea pig sera samples are applied to the ELISA plates with 10 µl sera and 90 µl PBS for peroxidase, and 1:50 dilutions of sera for cellulase and lipase, incubated for 1 hour and washed 3 times with PBS Tween20.

10

Then goat anti-guinea pig IgG<sub>1</sub> and 0.5% BSA 1:4000 in PBS buffer (Nordic Immunology 44-682) is applied to the plates, incubated for 1 hour and washed with PBS Tween20.

15 Alkaline phosphatase marked rabbit anti-goat 1:8000 is applied and incubated for 1 hour, washed 2 times in PBS Tween20 and 1 time with diethanol amine buffer.

The marked alkaline phosphatase is developed using p-nitrophenyl phosphate for 30 minutes at 37°C and stopped with calcium/sodium buffer comprising EDTA (pH 10) and read at OD 405/650 using a ELISA reader.

Double blinds are included on all ELISA plates.

25

Positive and negative sera values are calculated as the average blind values added 2 times the standard deviation. This gives an accuracy of 95%.

30 The test is described more thoroughly in APR 95255001, ED-9516670 available on request from Novo Nordisk A/S.

#### EXAMPLES

##### 35 EXAMPLE 1

Allergenicity trails with modified peroxidase

Dunkin Hartley guinea pigs were exposed to 1.0 µg purified monomer *Coprinus cinerea* peroxidase (guinea pig 21-30) and 1.0 µg modified dextran-peroxidase A (guinea pig 31-40) and dextran-peroxidase B (guinea pig 41-50) by intratracheal dosage as described ED-9516670 available on request from Novo Nordisk A/S.

All guinea pigs were tested for the production of IgG<sub>1</sub> (indicating an allergic response) during 8 weeks using the ELISA procedure described above.

Figure 1 shows the number of Dunkin Hartley guinea pigs found IgG<sub>1</sub> positive during the trial period.

It can be seen from Figure 1 the number of guinea pigs being IgG<sub>1</sub> positive at any time during the trial period is reduced for both dextran-peroxidase A and B in comparison to the monomer peroxidase. This proves that the allergenicity of polypeptides can be reduced by coupling to a suitable polymeric carrier molecule such as dextran.

#### Example 2

##### Allergenicity trails with modified lipase

The trails described in Example 1 were repeated, except that the Dunkin Hartley guinea pigs were stimulated intratracheally with either 1 µg purified lipase or 1 µg purified modified lipase.

All guinea pigs were tested for the production of IgG<sub>1</sub> (indicating an allergic response) during 10 weeks using the ELISA procedure described above.

As can be seen from Figure 2 the modified lipase has a reduced allergenicity.

### Example 3

#### Allergenicity trails with modified cellulase

The trails described in Example 1 were repeated, except that  
5 the Dunkin Hartley guinea pigs were stimulated intratracheally  
with either 1 µg purified cellulase or 1 µg purified modified  
cellulase.

All guinea pigs were tested for the production of IgG<sub>1</sub>  
10 (indicating an allergic response) during 10 weeks using the  
ELISA procedure described above.

As can be seen from Figure 3 the modified lipase has a reduced  
allergenicity.

15

As will be apparent to those skilled in the art in the light of  
the foregoing disclosure, many alterations and modifications  
are possible in the practice of this invention without depart-  
ing from the spirit or scope thereof. Accordingly, the scope of  
20 the invention is to be construed in accordance with the  
substance defined by the following claims.

**PATENT CLAIMS**

1. A conjugate with reduced allergenicity comprising a polymeric carrier molecule having two or more polypeptide molecules coupled thereto.
- 5 2. The conjugate according to claim 1 comprising polypeptide molecules coupled directly to the polymeric carrier molecule.
3. The conjugate according to claim 2 comprising polypeptide  
10 molecules coupled to the polymeric carrier molecules via a linker molecule.
4. The conjugate according to claim 3 comprising a polymeric  
15 carrier molecule having coupled thereto, via a covalent linkage, formed between one of the two vinyl groups of a divinyl sulfone, one or more polypeptide molecules.
5. The conjugate according to claims 1 to 4, wherein the polymeric carries molecule is selected from the group comprising  
20 natural and synthetic homo- and heteropolymers.
6. The conjugate according to claim 5, wherein the polymeric carrier molecule is selected from the group comprising synthetic polymeric molecules including Star-PEGs, Branched PEGs,  
25 poly-vinyl alcohol (PVA), poly-carboxyl acids, poly-(vinylpyrrolidone) and poly-D,L-amino acids.
7. The conjugate according to claim 5, wherein the polymeric carrier molecule is selected from the group comprising natural  
30 occurring polymeric molecules including dextrans, including carboxymethyl-dextrans, and celluloses such as methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, and hydrolysates of chitosan, starches, such as hydroxyethyl-starches, hydroxypropyl-starches, glyco-  
35 gen, agarose, guar gum, inulin, pullulan, xanthan gum carrageenin, pectin and alginic acid.

8. The conjugate according to any of claims 1 to 7, wherein the polypeptide is a protein of plant, animal or microbial, such as bacterial or fungal origin.
- 5 9. The conjugate according to any of claims 1 to 8, wherein the polypeptide is an anti-microbial polypeptide.
10. The conjugate according to claims 8 and 9, wherein the polypeptide has biologically activity.
- 10 11. The conjugate according to any of claims 8 to 10, wherein the polypeptide is an enzyme.
12. The conjugate according to claim 11, wherein the enzyme is
- 15 a protease, a lipase, a transferase, a carbohydrase, an oxidoreductase, or a phytase.
13. The conjugate according to claims 11 and 12, wherein the enzyme has a molecular weight ( $M_r$ ) of from about 4 kDa to 200
- 20 kDa, preferably 15 kDa to 150 kDa, especially 20 to 100 kDa.
14. The conjugate according to any of claims 1 to 13, wherein the molecular weight of the polymeric carrier molecule lies between 1 kDa and 10,000 kDa, preferably between 2 kDa and
- 25 5,000 kDa, especially between 5 kDa and 500 kDa.
15. The conjugate according to any of claims 1 to 14, wherein the total molecular weight ( $M_r$ ) of the conjugated molecule lies between 50 kDa and 40,000 kDa, preferably between 100 kDa and
- 30 1,000, especially 200 kDa and 500 kDa.
16. The conjugate according to claims 1 to 15, wherein between 1 and 60, preferably 2 and 40, especially 3 and 20 polypeptide molecules are coupled to one polymeric carrier.
- 35 17. The conjugate according to claims 1 to 16, wherein two or more different polypeptide molecules are coupled to each poly-

meric carrier molecule.

18. The conjugate according to claim 17, wherein said polypeptides exhibit at least two different activities, such as  
5 enzyme activities.

19. The conjugates according to claim 17, comprising one or more enzyme molecules and one or more ligands molecules.

10 20. The conjugates according to claim 17, comprising one or more antibody molecules and one or more inhibitor molecules.

21. The conjugate according to claim 17, comprising one or more receptor molecules and one or more antibody molecules.

15

22. A process of producing polypeptide molecules with reduced allergenicity comprising the steps of

- i) activating a polymeric carrier molecule, and
- ii) reacting one or more polypeptide molecules with said activated polymeric carrier molecule under conditions suitable for  
20 conjugation, and
- iii) blocking of residual active groups on the conjugate.

23. The process according to claim 22, comprising the steps of  
25 a) activating a polymeric carrier molecule by coupling thereto a reactive moiety, and  
b) reacting two or more polypeptide molecules with said activated polymeric carrier molecule.

30 24. The process according to claim 23 wherein the activation of the polymeric carrier molecule in step a) is performed by covalently linking thereto a reactive moiety derived from divinylsulfone.

35 25. The process according to any of claims 22 to 24, wherein the polymeric carrier molecule is a molecule according to claims 5 to 7.

26. The process according to any of claims 22 to 25, wherein the polypeptide molecules are a molecule according to any of claims 8 to 13.

5

27. The process according to any of claims 22 to 26, wherein the polymeric carrier molecule are coupled with the polypeptide molecules via an amino group ( $-NH_2$ ), a hydroxy groups ( $-OH$ ), a carboxylic acid group ( $-COOH$ ) or a thiol group on the polypeptide molecules.

10

28. A composition comprising a conjugate according to any of claims 1 to 21.

29. The composition according to claim 28, wherein the composition further comprises polypeptides, proteins and/or enzymes and/or ingredients normally used in detergents, including soap bars, household articles, agrochemicals, personal care products, including cleaning preparations for contact lenses and skin and hair cleaning preparations, cosmetics, toiletries, pharmaceuticals, such as oral and dermal pharmaceuticals, composition for treating textiles, compositions for cleaning hard surfaces, and compositions used for manufacturing food and feed.

20

25

30. Use of conjugated molecules according to any of claims 1 to 21 or compositions according to claims 28 and 29, for reducing the allergenicity of industrial products.

31. The use according to claim 30 where the industrial product is a personal care product.

30

32. The use according to claim 31 in hair care or hair treatment products, such as shampoo, balsam, hair conditioners, hair waving compositions, hair dyeing compositions, hair tonic, hair liquid, hair cream, shampoo, hair rinse, hair spray.

35



33. The use according to claim 31 in oral care products.

34. The use according to claim 33 in dentifrices, mouth washes, chewing gums.

5

35. The use according to claim 31, in skin care products, such as in skin cream, skin milk, cleansing cream, cleansing lotion, cleansing milk, cold cream, cream soap, nourishing essence, skin lotion, milky lotion, calamine lotion, hand cream, powder soap, transparent soap, sun oil, sun screen, shaving foam, shaving cream, and baby oil.

10

36. The use according to claim 31 in cosmetics, such as lipstick, lip cream, creamy foundation, face powder, powder eye-shadow, powder, foundation, make-up base, essence powder, whitening powder.

15

37. The use according to claim 31 for contact lenses hygiene products, such as contact lenses cleaning and disinfection products.

20

38. The use according to claim 30 in detergents, such as washing powder.

25 39. The use according to claim 38 in liquid detergents.

40. The use according to claim 38 in dishwash detergents.

30 41. The use according to claim 38 in soap, soap bars, liquid soap.

42. The use according to claim 30, in oral and dermal pharmaceuticals.

35 43. The use according to claim 30, in food or feed, including baking products.

44. The use according to claim 30, in products for processing textiles.

45. The use according to claim 30, in compositions for cleaning  
5 hard surfaces.

1/3

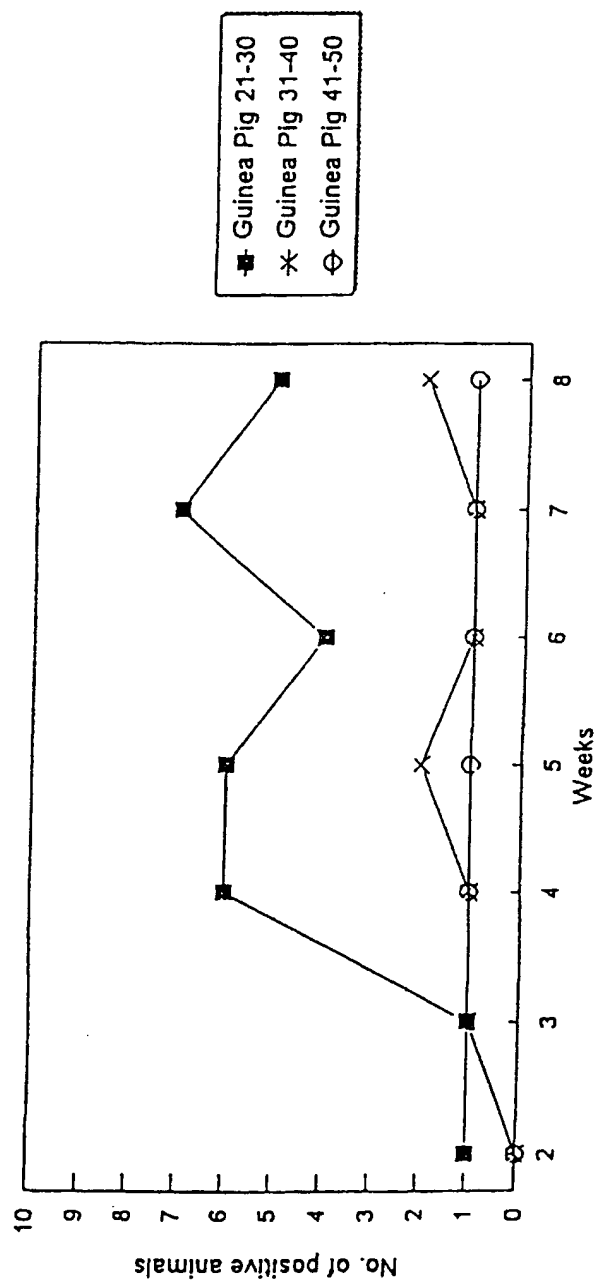


Fig. 1

2/3

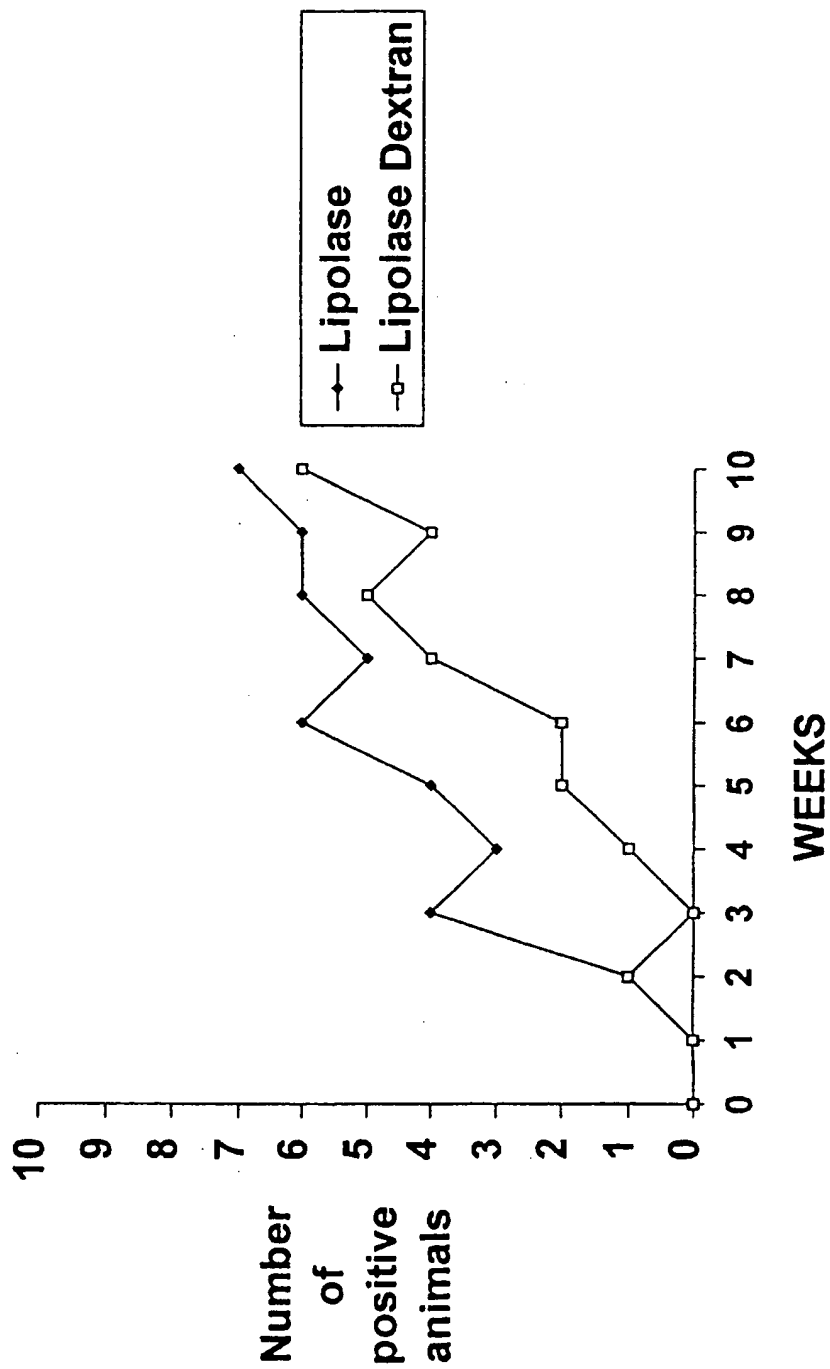


Fig. 2

3/3

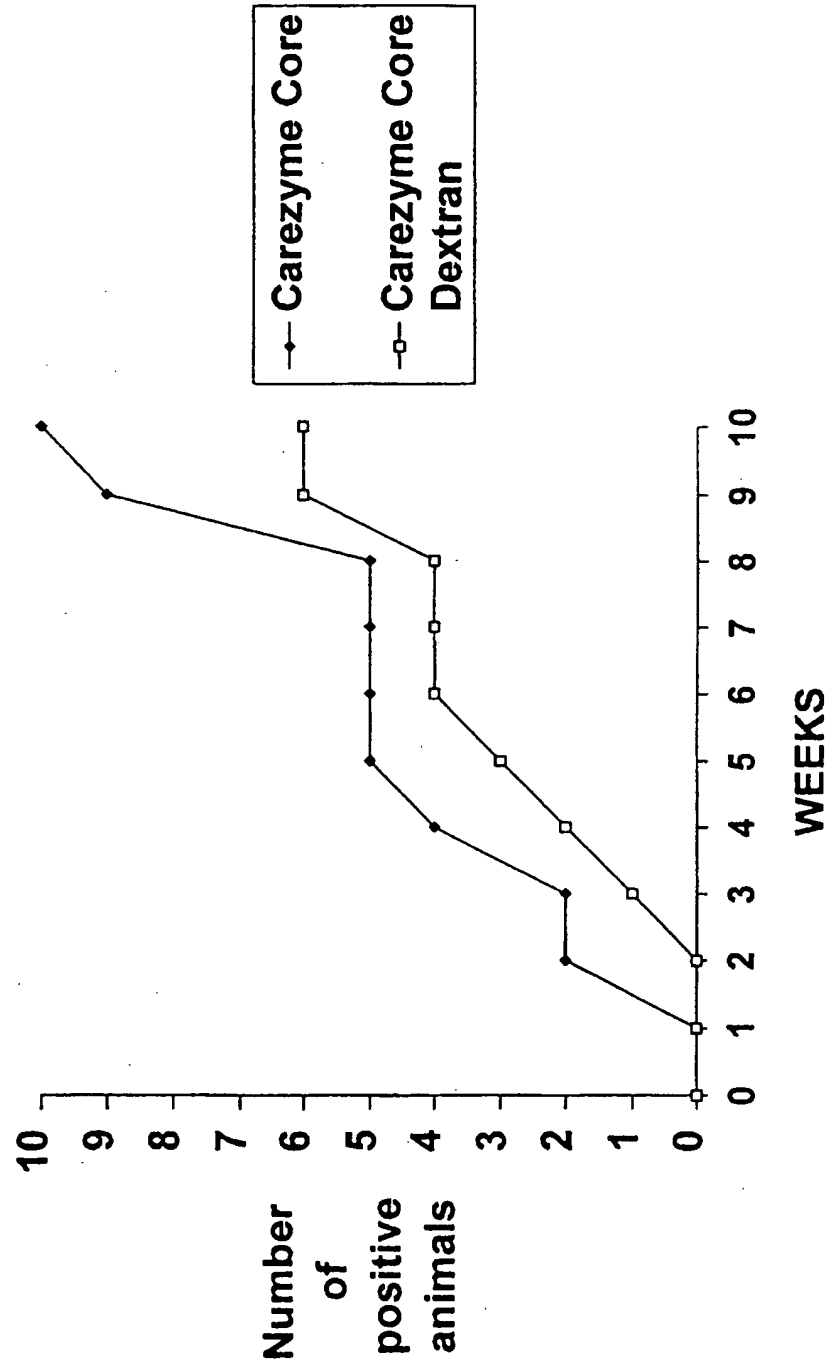


Fig. 3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00051

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/96, C12N 11/08, C07K 17/08, C11D 3/386, A61K 7/48, A61K 47/48  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, A61K, C11D, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, WPI, BIOSIS, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9617929 A1 (NOVO NORDISK A/S), 13 June 1996 (13.06.96) --	1-19,22-45
P,X	WO 9640791 A1 (NOVO NORDISK A/S), 19 December 1996 (19.12.96), page 7, line 1 - page 10, line 2 --	1-19,22-45
X	US 5133968 A (HIROSHI NAKAYAMA ET AL), 28 July 1992 (28.07.92), column 2, line 26 - line 64; column 7, line 38 - column 8, line 33 --	1-19,22-45

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance  
 "E" earlier document but published on or after the international filing date  
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 July 1997

Date of mailing of the international search report

16 -07- 1997

Name and mailing address of the ISA/  
 Swedish Patent Office  
 Box 5055, S-102 42 STOCKHOLM  
 Facsimile No. +46 8 666 02 86

Authorized officer

Anneli Jönsson  
 Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00051

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4179337 A (FRANK F. DAVIS ET AL), 18 December 1979 (18.12.79), column 2, line 26 - column 3, line 50, claims  --	1-19,22-45
X	EP 0183503 A2 (BEECHAM GROUP PLC), 4 June 1986 (04.06.86), page 1, line 24 - line 38; page 3, line 13 - page 4, line 30, claims  --	1-19,22-45
X	US 5006333 A (MARK SAIFER ET AL), 9 April 1991 (09.04.91), column 2, line 48 - line 64; column 3, line 45 - line 56; column 3, line 59 - column 4, line 4, abstract, abstract  --	1-19,22-45
X	US 5230891 A (HIROSHI NAKAYAMA ET AL), 27 July 1993 (27.07.93), column 2, line 40 - line 68, examples, claim 1  --	1-19,22-45
A	EP 0223221 A2 (BOEHRINGER MANNHEIM GMBH), 27 May 1987 (27.05.87), page 2, line 25 - line 40  -- -----	1-19,22-45

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00051

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next page

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-19, 22-45

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00051

## I. Claims 1-19, 22-45

Conjugate comprising a polymeric carrier molecule having two or more polypeptide molecules thereto. A process for producing the conjugate, a composition comprising the conjugate and the use of the conjugate for reducing the allergenicity of industrial products.

## II. Claims 20 and 28,30 (partially)

Conjugat comprising the polymeric carrier molecule having one or more antibody molecules and one or more inhibitor molecules. A composition comprising the conjugate and the use of conjugated molecules.

## III. Claims 21 and 28,30 (partially)

Conjugate comprising the polymeric carrier molecule having one or more receptor molecules and one or more antibody molecules. A composition comprising the conjugate and the use of conjugated molecules.

According to PCT Rule 13.2, a unity of inventions is fulfilled only when there is a "technical relationship" among the inventions involving one or more of the same or corresponding "special technical relationship features". In the instant groups I-III a "technical relationship" corresponding to the "special technical relationship features" is lacking because the special feature for groups I-III are:

Group I: The polypeptide is an enzyme. The description discloses only the use (industrial) of an enzyme-conjugate;

Group II: The polypeptides are an antibody molecule and an inhibitor molecule;

Group III: The polypeptide are a receptor molecule and an antibody molecule.

A single general inventive concept can not be established among the different inventions.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/DK 97/00051

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9617929 A1	13/06/96	AU 4114496 A	26/06/96
WO 9640791 A1	19/12/96	AU 5893696 A	30/12/96
US 5133968 A	28/07/92	US 5230891 A	27/07/93
US 4179337 A	18/12/79	CA 1033673 A	27/06/78
		CH 616942 A	30/04/80
		DE 2433883 A,C	05/02/76
		FR 2313939 A,B	07/01/77
		GB 1469472 A	06/04/77
		JP 1152589 C	30/06/83
		JP 50042087 A	16/04/75
		JP 56023587 B	01/06/81
		NL 7409770 A	22/01/75
		SE 441753 B,C	04/11/85
		SE 7409366 A	21/01/75
EP 0183503 A2	04/06/86	AU 5044585 A	17/07/86
		DE 3586313 A	13/08/92
		JP 2545339 B	16/10/96
		JP 6279318 A	04/10/94
		JP 7017517 B	01/03/95
		JP 61155333 A	15/07/86
		US 4935465 A	19/06/90
US 5006333 A	09/04/91	US 5080891 A	14/01/92
		US 5283317 A	01/02/94
		US 5468478 A	21/11/95
		AU 608901 B	18/04/91
		AU 2326888 A	01/03/89
		BR 8108745 A	06/07/82
		CA 1309046 A	20/10/92
		CN 1020458 B	05/05/93
		CN 1031088 A	15/02/89
		DE 3876997 A	04/02/93
		EP 0377613 A,B	18/07/90
		SE 0377613 T3	
		IE 64284 B	26/07/95
		JP 2504341 T	13/12/90
		JP 2515389 B	10/07/96
		SU 1776275 A	15/11/92
		WO 8901033 A	09/02/89
US 5230891 A	27/07/93	US 5133968 A	28/07/92

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

PCT/DK 97/00051

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0223221 A2	27/05/87	SE 0223221 T3	
		DE 3541186 A	27/05/87
		DE 3684879 A	21/05/92
		JP 1505414 C	13/07/89
		JP 62126977 A	09/06/87
		JP 63055918 B	04/11/88
		US 4910135 A	20/03/90
<hr/>			